6-1-1968

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THIN-LAYER CHROMATOGRAPHIC SEPARATIONS OF CYCLOKETONE 2,4-DINITROPHENYLHYDRAZONES

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JUNE, 1968

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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The author would like to dedicate this work to his wife, Prudence H. Slentz, whose many sacrifices contributed much in making it possible.

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ACKNOWLEDGEMENTS

The author would like to give his sincere thanks to Dr. Robert E. Gilman for his helpful advice and generous assistance throughout the course of this work, and in the preparation of this thesis.

The author would also like to express his appreciation to the members of his committee, Dr. Earl Krakower, Dr. Jerry Adduci, and Mr. Homer Imes.

Special thanks go to Dr. K. Thomas Finley who introduced the author to this problem and who was instrumental in guiding the early work upon which this thesis is based.

Acknowledgements would be incomplete if the author did not express his great debt of gratitude to the following Wheatland-Chili High School graduates who over the past few years contributed much of their time, effort, and energy in attacking this problem:

Virginia Birch, John Fay, Linda Phaff, Timothy Quigley, Edward Ritz, Marc Robertson, Donald Schaeffer, Shelly Williams, William Van Duser.

ABSTRACT

Ten cycloketone 2,4-dinitrophenylhydrazones have been chromatographed by thin-layer techniques using Eastman Chromagram sheets coated with silica gel and developed with benzene in Eastman sandwich apparatus.

Good separations of all test compounds from binary mixtures with the standard cyclohexanone 2,4-dinitrophenylhydrazone are reported as well as good separations of more complex mixtures of the test compounds.

Lack of precision is noted despite a rigorous standardized procedure. Evidence is offered that chromagram sheets differ in adsorbent activity.

THIN-LAYER CHROMATOGRAPHIC SEPARATIONS OF CYCLOKETONE 2,4-DINITROPHENYLHYDRAZONES

INTRODUCTION:

During the summer of 1964, the author (a secondary school chemistry teacher) and two of his students participated in a National Science Foundation sponsored CCSS research institute at Rochester Institute of Technology. The work during that summer consisted of collecting data on R_F values for a number of carbonyl 2,4-dinitrophenylhydrazones (2,4-DNPHs) using paper chromatography according to a standardized technique[10]. Included in the compounds studied were eight cycloketone 2,4-DNPHs whose R_F values were so close that good separations were not possible.

The author and several of his students, working as part of an Independent Study Program in Science at the Wheatland-Chili High School, decided to examine other systems, particularly thin-layer chromatography, in an attempt to effect separations of these compounds.

Other investigators have applied a variety of thin-layer chromatography techniques to a large number of carbonyl compounds. As early as 1961, Rosmus and Deyl[18] reported the separation of several carbonyl 2,4-DNPHs, including that of cyclohexanone, on glass plates coated with aluminum oxide adsorbent and using a benzene-hexane mixture as a developing solvent. In that same year, Dhont and DeRooy[7] separated several aromatic carbonyl 2,4-DNPHs on glass plates spread with a layer of silica gel and developed in a benzene-petroleum ether mixture. In that same study, Dhont and DeRooy also determined the R_B values (R_F values relative to the R_F of Butter Yellow) of straight chain aliphatic aldehydes as a function of chain length. In 1962 Anet[1] used silica gel plates developed in a toluene-

ethyl acetate mixture to separate the 2,4-DNPHs of a number of hydroxy-carbonyl compounds. In 1963 Urbach[22] separated the members of several homologous series of aliphatic aldehydes and ketones using a partition system between 2-phenoxyethanol supported on kieselguhr G and light petroleum ether. Also in 1963, Nano and Sancin[17] found R_F values for several aliphatic carbonyl 2,4-DNPHs using an alumina adsorbent with a cyclohexane-nitrobenzene solvent mixture.

In more recent years, several investigators[2,3,6,8,11,12,19,23] have used thin-layer techniques to separate or identify the 2,4-DNPHs of a large number of aldehydes and ketones. Most of these workers used glass plates coated with silica gel, alumina, or kieselguhr and many different solvent mixtures for development. Davidek[4,5] carried on successful separations of the 2,4-DNPHs of aliphatic aldehydes and ketones on starch impregnated with dimethyl formamide developed in saturated hexane. Edwards[9] used a TLC technique consisting of a stationary phase of n-undecane on kieselgel G and a mobile phase of methanol-water to separate the stereoisomers of the 2,4-DNPHs of some aliphatic aldehydes and unsymmetrical ketones. Meijboom[15] used glass plates coated with kieselguhr G impregnated with carbowax 400 toseparate a variety of saturated and unsaturated aldehyde 2,4-DNPHs.

In all of these works, only scattered reference is made to separation of cycloketone 2,4-DNPHs. A most comprehensive study of TLC separations of carbonyl 2,4-DNPHs was made by Brümmer and Müller-Penning[2] in which they investigated nearly 250 compounds using a kieselguhr G adsorbent with a developing solvent of petroleum ether-chloroform-ethyl acetate(30:3:1). Reported among the many R_B values (R_F relative to Butter Yellow) from this study were those of five

cycloketone 2,4-DNPHs : cyclopentanone(0.72), cyclohexanone(0.85), cycloheptanone(0.96), cyclooctanone(1.00) and cyclopentadecanone(1.20).

Denti and Luboz[6] have reported R_{FOR} values (R_F values relative to the R_F of formaldehyde) for the 2,4-DNPHs of cyclopentanone and cyclohexanone using adsorbent layers of silica gel G and alumina G (both alone and impregnated with silver nitrate) with four different solvent mixtures. Zamojski and Zamojska[23] also determined R_{FOR} values for these two compounds in a study which included a large number of aliphatic aldehydes and ketones using silica gel with a benzene developer. Both groups obtained good separations for these two compounds. Jart[12] included R_F values for cyclopentanone and cyclohexanone 2,4-DNPHs along with those for 70 other carbonyl compounds. Sloot[21] used alumina impregnated with silver nitrate to separate several 2,4-DNPHs including those of cyclopentanone, cyclohexanone, 3-methylcyclopentanone, and 2-methylcyclopentanone.

As far as can be determined, no other studies have included a homologous series of aliphatic cycloketone 2,4-DNPHs as does the study upon which this report is based.

RESULTS:

Comparison of R_F values seems more meaningful when they are reported relative to an internal standard. Because cyclohexanone is the most readily available cycloketone, its 2,4-DNPH was chosen as a standard. A $R_{(Standard)}$ value (R_S) was then defined as:

$$R_S = \frac{\text{migration distance of cycloketone 2,4-DNPH}}{\text{migration distance of cyclohexanone 2,4-DNPH}}$$

The mean R_F values and the mean R_S values for each of the ten cycloketone derivatives included in this study are reported in Table I with their standard deviations. These values are also presented graphically in Figure 2 and Figure 3. Each of the values in the table represents the mean for 20 separate runs. Only those sheets on which the R_F value of the standard was 0.22 or less were included in this summary (see discussion).

TABLE I

R_F AND R_S VALUES FOR CYCLOKETONE 2,4-DNPH s

2,4-DNPH of	$R_F \pm \sigma$	$R_S \pm \sigma$
cyclobutanone	0.13 ± .02	0.61 ± .02
cyclopentanone	0.15 ± .01	0.73 ± .02
cyclohexanone	0.20 ± .02	1.00
cycloheptanone	0.28 ± .02	1.41 ± .04
cyclooctanone	0.28 ± .02	1.43 ± .04
cyclononanone	0.33 ± .02	1.71 ± .05
cyclodecanone	0.40 ± .04	2.06 ± .08
cycloundecanone	0.44 ± .04	2.23 ± .11
cyclododecanone	0.49 ± .04	2.53 ± .12
cyclopentadecanone	0.53 ± .04	2.70 ± .12

Table II shows the R_F and R_S values from 2 consecutive sheets that were activated, spotted and developed simultaneously.

TABLE II

R_F AND R_S VALUES FROM CONSECUTIVE SHEETS

2,4-DNPH OF	SHEET #73		SHEET #74	
	R_F	R_S	R_F	R_S
cyclobutanone	0.14	0.64	0.25	0.69
cyclopentanone	0.16	0.75	0.29	0.83
cyclohexanone	0.20	1.00	0.36	1.00
cycloheptanone	0.30	1.42	0.47	1.29
cyclooctanone	0.29	1.39	0.49	1.28
cyclononanone	0.34	1.72	0.53	1.46
cyclodecanone	0.41	2.03	0.57	1.65
cycloundecanone	0.45	2.23	0.62	1.75
cyclododecanone	0.51	2.50	0.65	1.81
cyclopentadecanone	0.54	2.67	0.70	1.95

The situation illustrated in Table II was encountered again and again in the total of 40 runs made in this series. On those sheets on which the R_F value of the standard was high, the spread of R_S values seemed to be markedly decreased. Since the variation of R_F values of the standard was great, even among sheets run at the same time under the same conditions, a study was made of the R_S value of each compound as the R_F value of the standard varied. Table III is a tabulation of such data for cyclopentadecanone 2,4-DNPH.

TABLE III

VARIATION IN R_S VALUES FOR CYCLOPENTADECANONE 2,4-DNPH (R_S(#15)) WITH VARYING R_F VALUE OF STANDARD (R_F (#6))

R_F(#6)	R_S(#15)	R_F(#6)	R_S(#15)
0.16	2.95	0.23	2.41
0.17	2.83	0.23	2.58
0.18	2.78	0.23	2.53
0.18	2.87	0.24	2.48
0.18	2.83	0.24	2.47
0.18	2.67	0.24	2.63
0.18	2.79	0.24	2.38
0.19	2.75	0.24	2.43
0.20	2.76	0.25	2.59
0.20	2.71	0.26	2.30
0.20	2.56	0.27	2.30
0.20	2.67	0.28	2.24
0.20	2.66	0.28	2.30
0.20	2.50	0.30	2.13
0.21	2.66	0.31	2.19
0.21	2.64	0.33	2.09
0.21	2.70	0.36	1.95
0.21	2.52	0.38	1.85
0.22	2.63	0.41	1.81
0.22	2.60		

Similar comparisons of R_S values of other test compounds with changing R_F of the standard show similar decreases in R_S values, with the exception of cyclobutanone and cyclopentanone 2,4-DNPHs which increase slightly with increasing R_F of the standard.

DISCUSSION:

Precision of Results

Throughout the experimental procedure, chromagram sheets were activated, spotted, and developed in groups of four. It was expected, therefore, that the precision within such a group should be very high. In fact, however, considerable deviation was found not only from group to group, but also from sheet to sheet within a group (see Table II).

Table III shows the general inverse relationship between the R_S value of cyclopentadecanone 2,4-DNPH and the R_F value of the standard cyclohexanone 2,4-DNPH. Figure 4 shows a graph of the R_S value for this compound plotted against the reciprocal of the square root of the R_F of the standard.

The author concludes that despite the rigorous standardized procedure followed, differences in R_F and R_S values from sheet to sheet are caused by differences in the chromagram sheets themselves, perhaps variations in particle size, layer thickness, or pH. In a recent paper, Jolliffe and Shellard[13] also found a high variance ratio between the values taken from different chromagram sheets from the same pack and suggested that the lack of reproducibility may have been due to a lack of constancy in the pH of the silica gel layer which they found to vary from 6.5 to 4.0.

Whatever the reason for variation in values, the R_F value of the standard seems to reflect rather accurately the adsorbent activity of the sheet. For these reasons, in compiling the data for Table I, only those sheets were considered on which the R_F value of the standard was at or below the arbitrary value of 0.22, which included the most active half of the 40 sheets developed in this study.

Separations

Each member of the series of compounds tested separated clearly from the standard. In addition, all compounds whose structure varied by two or more carbon atoms were separable. This was illustrated experimentally by the good separations of the complex mixtures composed of all the odd-numbered or of all the even-numbered cycloketone derivatives (see Figures I and IA). Adjacent members of the series can be separated with the exception of the derivatives of cycloheptanone and cyclooctanone, cyclodecanone and cycloundecanone, and cycloundecanone and cyclododecanone.

Of special interest were the R_F and R_S values for the 2,4-DNPHs of cycloheptanone and cyclooctanone. Within experimental error, these values were identical. It is noted that in their comprehensive study of TLC separations of carbonyl 2,4-DNPHs, Brümmer and Müller-Penning[2] also obtained very similar values for these two compounds (0.96 and 1.00), although these authors give no indication of the precision of their results. In the present study, melting point determinations and NMR spectra were made to establish definitely the identities of these two compounds. From Figures 2 and 3, it appears that the values for the cycloheptanone derivative are elevated with respect to the other test compounds. The author is unable to suggest a reason for this elevation of values.

EXPERIMENTAL

Materials:

All of the cycloketone 2,4-DNPHs used in this experiment were prepared by the method of Shriner, Fuson, and Curtin[20] and were recrystallized from methanol until the melting points agreed with those found in the literature[16,20].

Solvents used for recrystallization, spotting, and developing were all "reagent grade".

Solutions of the test compounds were spotted on 20 X 20 cm. Eastman Chromagram Sheets (Type 6061) with a stated silica gel coating thickness of 100 microns.

Benzene was used exclusively as the solvent for development.

Procedure:

Considerable effort was expended to insure standardization of procedure. The following steps were followed carefully in the collection of all data presented.

(1) Solutions of the 2,4-DNPHs used for spotting were made by dissolving 100 mg. of the test compound in 100 ml. of a chloroform-methanol (1:1) mixture. Since all the spotting solutions contained the same solvent mixture, the variable effect of different spotting solvents on spot mobility noted by Kidder and Dewey[14] was avoided.

(2) All chromagram sheets were activated for 60 minutes in a drying oven at 100-110°C. before spotting. Activated sheets were stored in a dessicator until spotted.

(3) Spots were applied to the 20 X 20 cm. activated chromagram sheets with a micropipet. Each spot contained 1.5 to 2.0 microliters of each compound in the spot. Spot diameter was kept below three mm.

Spots were located 20 mm. from the bottom edge of the sheet and 20 mm. from each side. The distance between adjacent spots was 16 mm. Each sheet contained spots of nine binary mixtures (the 2,4-DNPHs of the standard cyclohexanone and another of the cycloketones of the series), a spot of the standard alone, and a spot of one of two complex mixtures. The complex mixtures consisted of five compounds, the derivatives of all the odd-numbered cycloketones or the derivatives of all the even-numbered cycloketones. Two such sheets (#73 and #74) are illustrated after development in Figures I and IA.

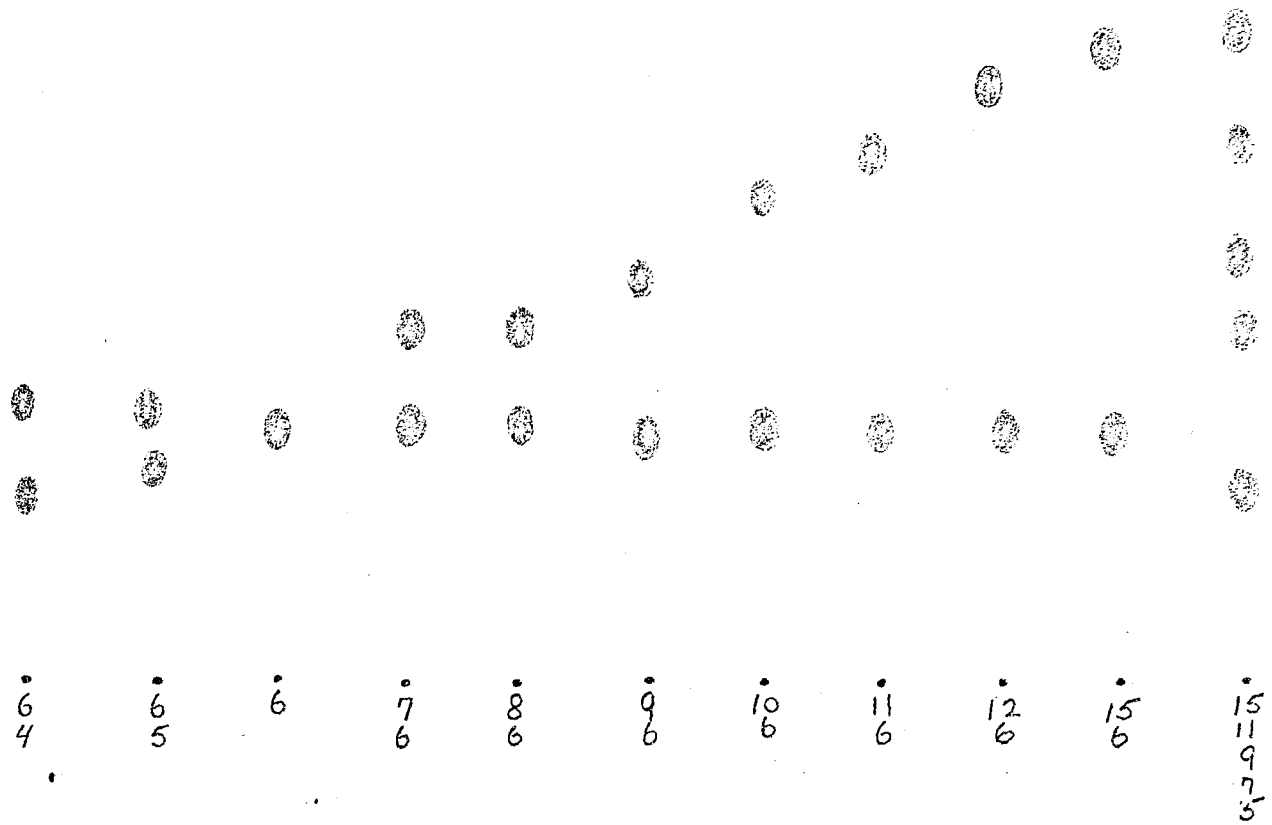
(4) Spotted sheets were heated for five minutes at 100-110°C. in a drying oven to insure complete evaporation of the spotting solvent. Spotted sheets not developed immediately were stored until used in a dessicator.

(5) The spotted sheets were developed in Eastman Chromagram Developing Apparatus (6071). Since four such sandwich-type chambers were available, four sheets were developed simultaneously. All development was over a distance of 150 $\pm$ 4 mm. using benzene as a developing solvent.

FIGURE 1

#73

Solvent Front

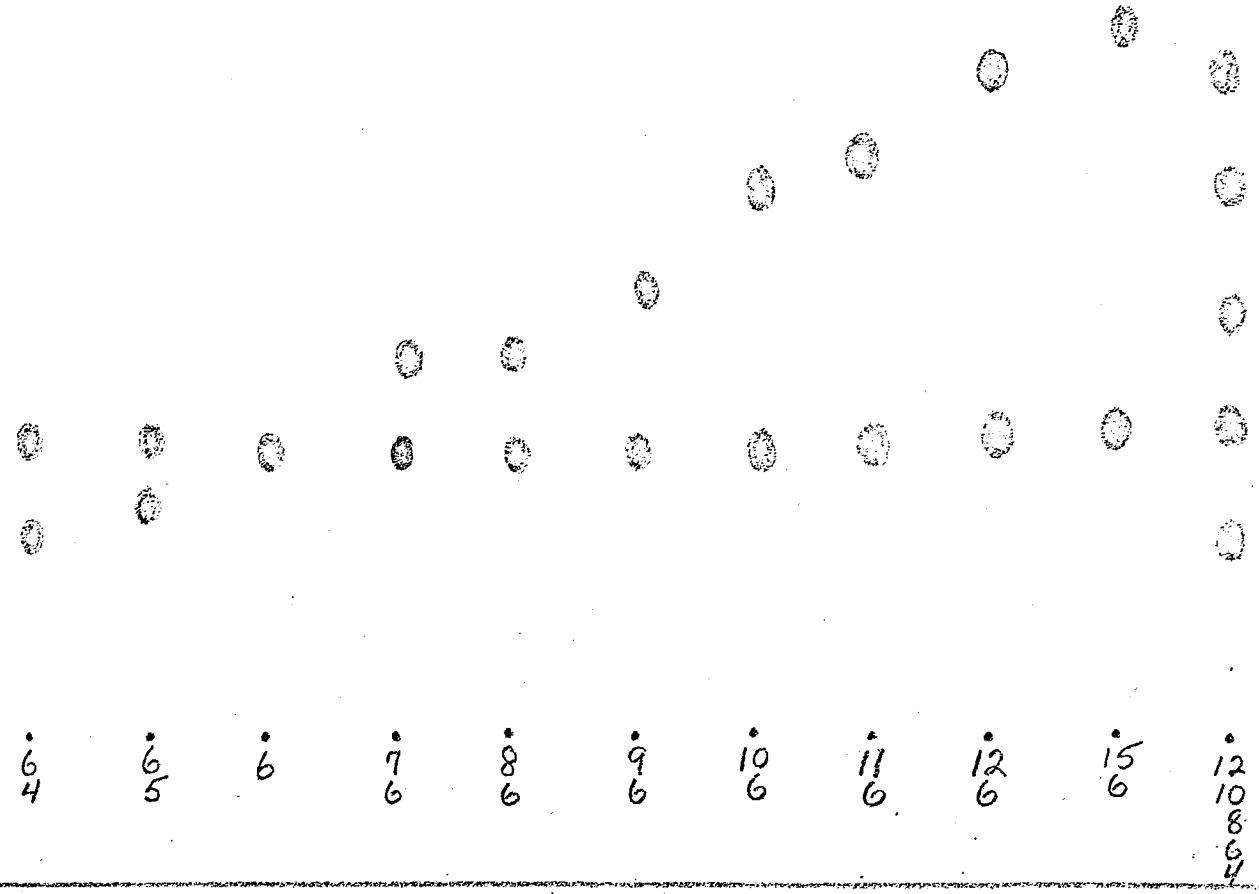


DEVELOPED SHEET ORIGINALLY SPOTTED WITH 2,4-DNPHs OF:

4	cyclobutanone	9	cyclononanone
5	cyclopentanone	10	cyclodecanone
6	cyclohexanone(standard)	11	cycloundecanone
7	cycloheptanone	12	cyclododecanone
8	cyclooctanone	15	cyclopentadecanone

FIGURE 1A

Solvent Front



DEVELOPED SHEET ORIGINALLY SPOTTED WITH 2,4--DNPHs OF:

4 cyclobutanone
5 cyclopentanone
6 cyclohexanone(standard)
7 cycloheptanone
8 cyclooctanone
9 cyclononanone
10 cyclodecanone
11 cycloundecanone
12 cyclododecanone
15 cyclopentadecanone

FIGURE 2

R_F VALUES OF CYCLOKETONE 2,4-DNPHs

VERSUS

NUMBER OF CARBONS IN CYCLOKETONE

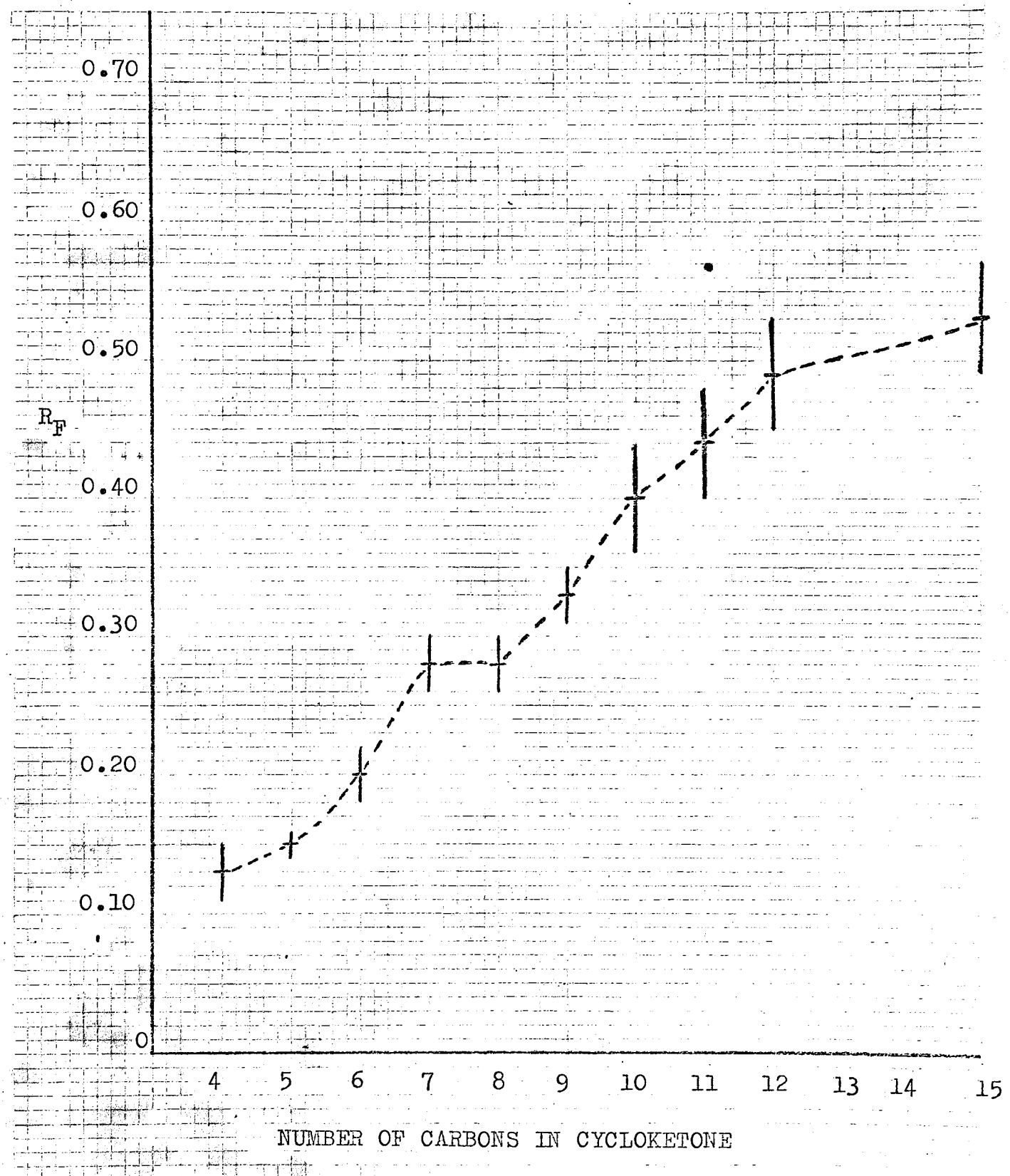


FIGURE 3

R_S VALUES OF CYCLOKETONE 2,4-DNPHs

VERSUS

NUMBER OF CARBONS IN CYCLOKETONE

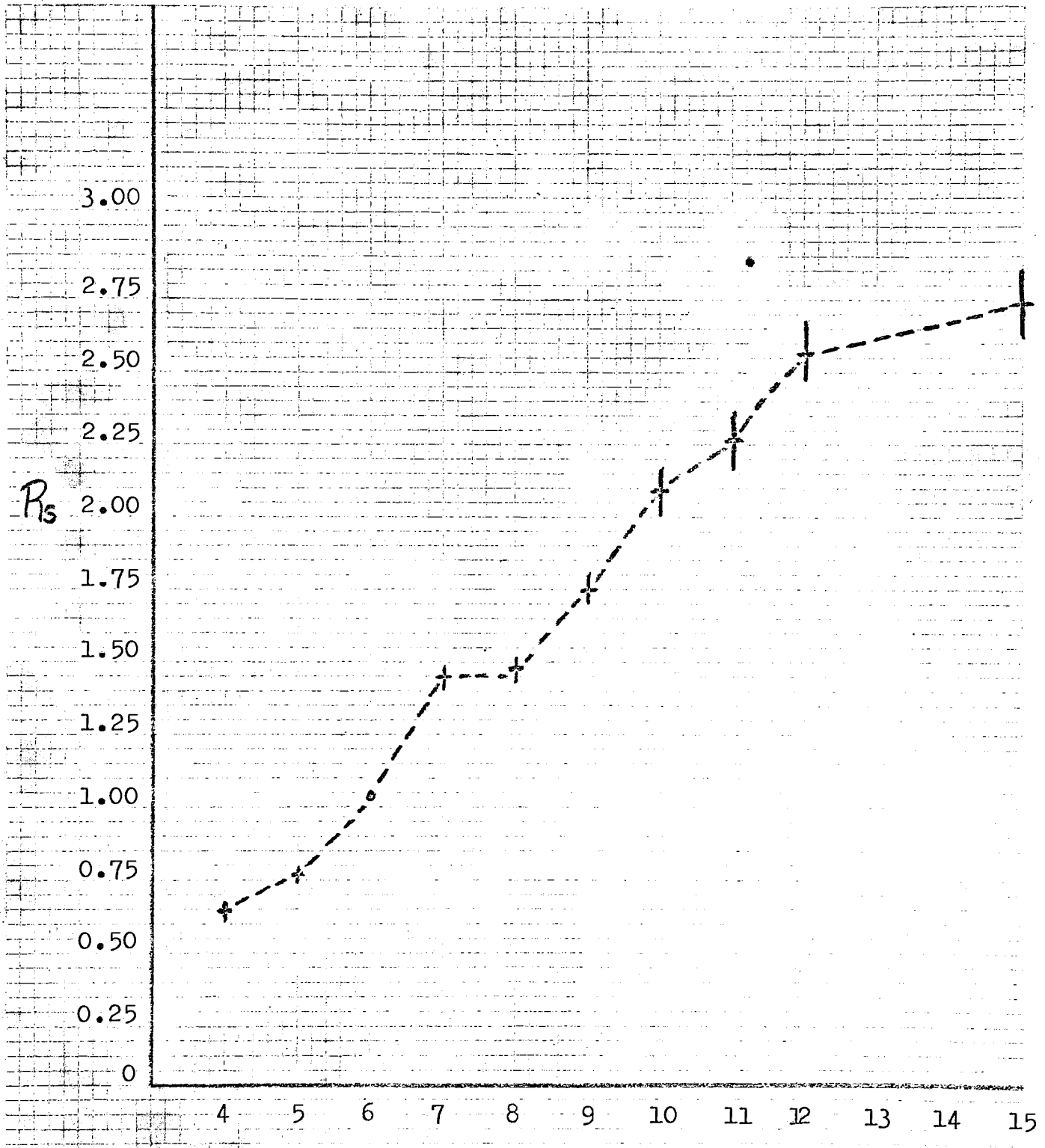
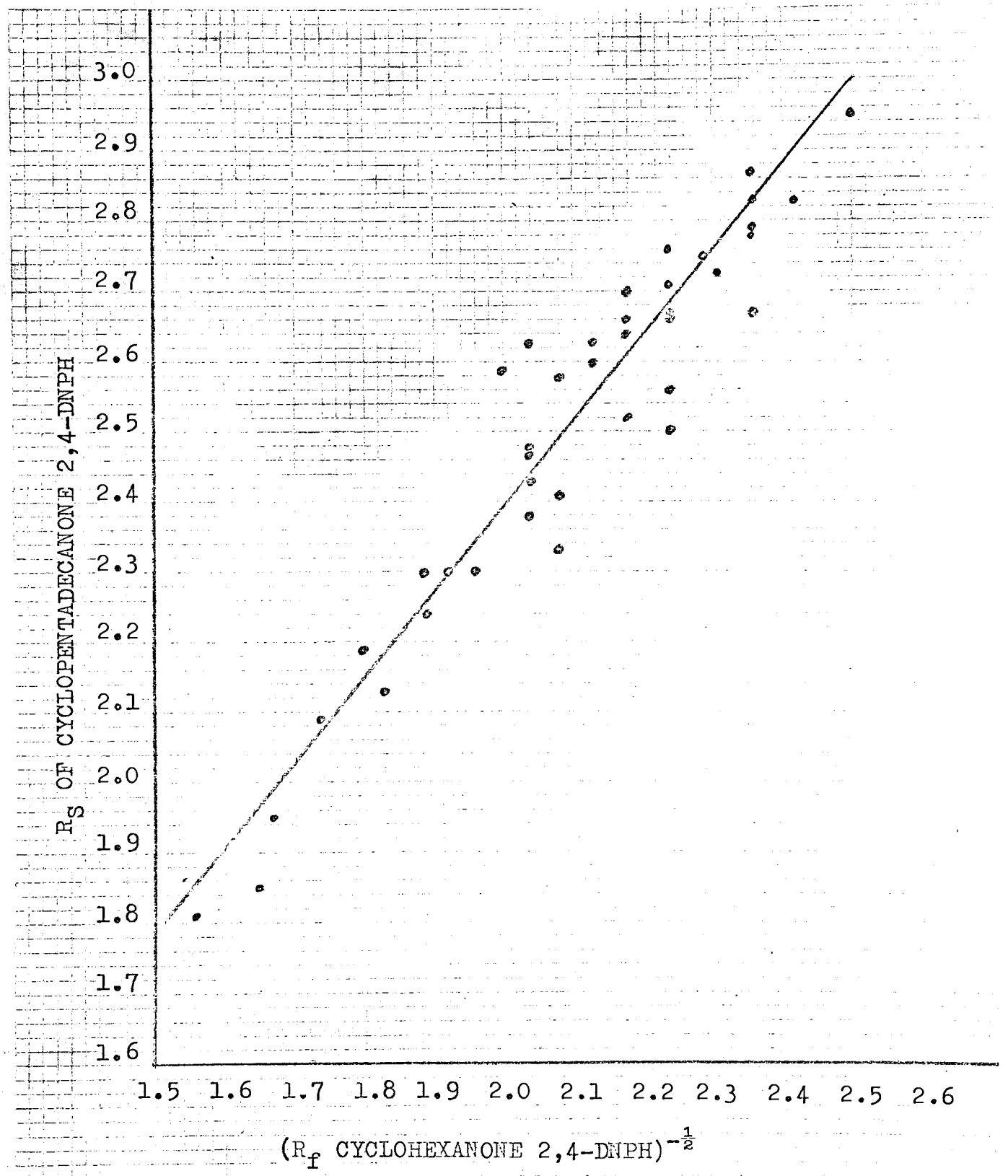


FIGURE 4

R_S CYCLOPENTADECANONE 2,4-DNPH

VERSUS

$(R_F$ CYCLOHEXANONE 2,4-DNPH$)^{-\frac{1}{2}}$



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